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Pyramiding QTL for multiple lateral branching in cucumber using inbred backcross lines

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Abstract Multiple lateral branching (MLB) is a quantitatively inherited trait associated with yield in cucumber (*Cucumis sativus* L.; $2n = 2x = 14$). Although quantitative trait loci (QTL) have been identified for MLB and QTL-marker associations have been verified by marker-assisted selection, the individual effects of these QTL have not been characterized. To test the effects of pyramiding QTL for MLB, molecular genotyping was utilized to create two sets (standard- and little-leaf types) of inbred backcross (IBC) lines possessing various numbers of QTL that promote branching. These IBC lines were evaluated for lateral branch number in two Wisconsin environments at three plant densities. Highly significant differences in the number of primary lateral branches were detected between spacings, leaf types, and lines, but not between locations. Lateral branch number decreased at higher plant densities in all genotypes, while genotype by environment and QTL by environment interactions were marginally non-significant. As the number of QTL increased among IBC lines, the number of branches did not generally change in the little-leaf lines, but decreased in the standard-leaf lines, demonstrating an epistatic effect related to genetic background during lateral branch development. The genomic location with the greatest effect on MLB was confirmed as the QTL that was previously mapped near the little-leaf locus (*ll*), while the addition of one specific QTL consistently decreased the number of lateral branches in standard-leaf lines. Although pyramiding QTL for MLB did not uniformly increase the number of lateral branches, pyramiding QTL in IBC lines allowed further characterization of individual QTL involved in MLB. Our results, coupled with those of previous studies indicate that lateral branch development in cucumber is determined by growing environment (i.e., plant spacing), genetic background, and QTL composition.

Keywords *Cucumis sativus* · Pyramiding · Multiple lateral branching · Inbred backcross lines · Quantitative trait loci

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Abbreviations

IBC	Inbred backcross
MAS	Marker-assisted selection
MLB	Multiple lateral branching
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RIL	Recombinant inbred lines

Introduction

Cucumber (*Cucumis sativus* var. *sativus* L.) is the fifth most widely grown vegetable crop worldwide (2,427,436 ha harvested in 2004) and ranks seventh in the US in area harvested (68,660 ha; FAOSTAT, 2005). Although the yield of US processing cucumber has reached a plateau in the last 20 years, evidence from several studies indicates that selection for multiple lateral branching (MLB) types (i.e., plants with several primary lateral branches) may increase cucumber yield (Fredrick and Staub 1989; Cramer and Wehner 1998, 1999, 2000a, b). In addition, highly-branched and determinate plant types are desirable for once-over machine harvest of US processing cucumber (Lower and Edwards 1986; Wehner 1989; Staub et al. 1992).

A feral relative of cucumber, *C. sativus* var. *hardwickii* (R) Alef. (hereafter referred to as *C. s.* var. *hardwickii*; Horst and Lower 1978), and a cucumber inbred line 'Little John' (line H-19; synom. AR 79-75; Goode et al. 1980) both possess a multiple lateral branching habit (7–12 primary lateral branches) not present in commercial cucumber. Multiple lateral branching in both sources is moderately heritable (heritabilities $\geq$0.61 with at least four genes) where four quantitative trait loci (QTL) explained 48–66% of the observed variation (R^2) in F_3 lines derived from Gy-7 (synom. G421; 1–2 primary lateral branches) and H-19 (Wehner et al. 1978; Serquen et al. 1997a, b; Fazio et al. 2003b). Using recombinant inbred lines (RIL) derived from the same parents, Fazio et al. (2003b) identified five QTL with a combined R^2 of 37–55%. In both QTL studies, one major QTL accounted for 32% (Fazio et al. 2003b) to 40% (Serquen et al. 1997b) of the phenotypic variation and mapped near the little-leaf locus (*ll*).

The QTL analyses of Gy-7 × H-19 derived populations (Serquen et al. 1997b; Fazio et al. 2003b) provided marker-QTL relationships that have been verified by marker-assisted selection (MAS) of MLB (Fazio et al. 2003a; Fan et al. 2006; Robbins 2006). The increase in the number of branches from MAS was comparable to phenotypic selection after two generations of backcrossing to Gy-7, the low branching parent (Fazio et al. 2003a). Likewise, two generations of MAS backcrossing after two cycles of phenotypic recurrent selection for MLB in a similar population continued to increase the number of lateral branches, and operated to fix favorable alleles that were not exploited by phenotypic selection (Fan et al. 2006).

Pyramiding QTL affecting the same trait has been utilized as a method to further characterize and validate individual QTL (Castro et al. 2003a, b; Richardson et al. 2006). Little is known about the effects and interactions among individual QTL involved in MLB in cucumber. Therefore, inbred backcross (IBC) lines possessing different numbers of QTL for MLB were created and evaluated at two locations and three plant densities to further characterize QTL involved in MLB in both little- (30–40 cm^2) and standard-leaf (>40 cm^2; Staub et al. 1992) plant types.

Materials and methods

Two parents (Gy-7 and H-19) previously used in cross-progeny QTL analyses (Serquen et al. 1997b; Fazio et al. 2003b) were utilized to create IBC lines with varying numbers of QTL for MLB. Six QTL affecting MLB were chosen for the development of IBC lines because of their relatively high LOD scores, R^2 percentages, and genetic effects (Table 1). Molecular markers (Table 2) were employed for introgression of these QTL to construct an array of IBC lines that varied in QTL number (Table 3). The effect of a specific QTL on MLB was estimated by comparing IBC lines that differed only by that QTL (Table 4). IBC lines with specific sets of QTL were compared in different growing environments to characterize individual QTL effects and to provide for an understanding of environmental factors governing MLB.

IBC line creation

Lines Gy-7 (standard-leaf type; *LL*) and H-19 (little-leaf type; *ll*) were crossed to create F_1 individuals that

Table 1 Quantitative trait loci (QTL) previously associated with multiple lateral branching (MLB) in cucumber that were used to create inbred backcross (IBC) lines

QTL	Recombinant inbred line analysis[a]										$F_{2:3}$ progeny analysis[b]			
	Hancock, WI 1999				Hancock, WI 2000			Utah 1999			Tifton, GA		Hancock, WI	
	Name[c]	LOD[d]	R^2 (%)[e]	Effect[f]	LOD	R^2 (%)	Effect	LOD	R^2 (%)	Effect	LOD	R^2 (%)	LOD	R^2 (%)
MLB1	*mlb1.4*	32.9	32.4	0.63	7.0	8.1	0.23	9.8	17.2	0.42	4.6	13.6	nd	nd
MLB2	*mlb1.1*	11.6	9.1	0.36	8.2	10.6	0.24	6.8	11.5	0.33	10.4	39.6	10.1	37.0
MLB3	*mlb6.2*	4.2	3.7	−0.17	3.7	4.3	−0.15	2.7	4.9	−0.18				
MLB4											nd	nd	3.3	11.0
MLB5	*mlb4.4*	3.0	1.7	0.17	4.6	4.6	0.37	2.7	3.3	0.20				
MLB6	*mlb6.1*	2.7	1.5	0.11	3.0	2.9	0.17	nd[g]	nd	nd				

[a] QTL identified by Fazio et al. (2003b)

[b] QTL identified by Serquen et al. (1997a)

[c] QTL name given by Fazio et al. (2003b)

[d] Log of likelihood ratio

[e] Percentage of the phenotypic variation explained

[f] The effect of the H-19 allele on the number of lateral branches

[g] nd = not detected

Table 2 Marker-QTL associations used for introgression of quantitative trait loci (QTL) for multiple lateral branching (MLB) to create inbred backcross (IBC) lines in cucumber

Linkage group[a]	Top flanking marker[b]	Distance (cM)[c]	QTL	Distance (cM)[c]	Bottom flanking marker[d]
1	OP-AD12-1	9.1	*MLB1*	3.7	*ll*[e]
1			*MLB2*	6.1	OP-AG1-1
6	L19-2-SCAR	6.3	*MLB3*	16.1	NR60
1	AJ6SCAR		*MLB4*[f]		BC523SCAR
4	CSWTAAA01	2.3	*MLB5*		
6	AK5SCAR	4.1	*MLB6*	2.0	M8SCAR

[a] The linkage group in the genetic map of Fazio et al. (2003b)

[b] The marker above the QTL in the genetic map of Fazio et al. (2003b)

[c] The genetic map distance between the marker and the QTL

[d] The marker below the QTL in the genetic map of Fazio et al. (2003b)

[e] The little-leaf gene (Pierce and Wehner 2000)

[f] *MLB4* was mapped between AJ6SCAR and BC523SCAR (Serquen et al. 1997b), which are separated by 5.1 cM (Fazio et al. 2003b)

were subsequently backcrossed to both parents resulting in BC_1 populations uniformly possessing standard leaves (Gy-7 as recurrent parent) and segregating for leaf type (H-19 as recurrent parent). Individuals from both BC_1 populations were phenotypically selected for high lateral branch number and leaf type in an open-field nursery at the University of Wisconsin Agricultural Research Station, Hancock, Wisc. [Plainfield loamy sand (Typic Udipsamment; sandy, mixed, mesic)], and then backcrossed to the recurrent parents to create little- and standard-leaf BC_2 and subsequently BC_3 families. These families were evaluated for MLB at Hancock in 2001, and 50 highly branched individuals (30 standard-leaf and 20 little-leaf) were selfed to create BC_2S_1 and BC_3S_1 families of each leaf type.

Table 3 QTL composition and mean number of branches in inbred backcross (IBC) lines of cucumber

IBC line[a]	Leaf type[b]	Quantitative trait loci[c]						Generation	Number of lateral branches per plant[d]		
		MLB1	*MLB2*	*MLB3*	*MLB4*	*MLB5*	*MLB6*		Hancock, Wisc.	Arlington, Wisc.	Mean
IBC-1246	Little	*MLB1*	*MLB2*		*MLB4*		*MLB6*	BC_2S_5	6.75	7.30	7.03
IBC-1236	Little	*MLB1*	*MLB2*	*MLB3*			*MLB6*	BC_2S_5	7.17	8.23	7.70
IBC-1356	Little	*MLB1*		*MLB3*		*MLB5*	*MLB6*	BC_2S_6	7.20	7.44	7.32
IBC-12456	Little	*MLB1*	*MLB2*		*MLB4*	*MLB5*	*MLB6*	BC_2S_5	7.13	6.77	6.95
IBC-12356	Little	*MLB1*	*MLB2*	*MLB3*		*MLB5*	*MLB6*	BC_2S_5	7.24	8.19	7.71
IBC-146	Little	*MLB1*			*MLB4*		*MLB6*	BC_2S_6	7.64	7.13	7.39
IBC-36	Standard			*MLB3*			*MLB6*	BC_3S_5	1.45	1.38	1.41
IBC-23	Standard		*MLB2*	*MLB3*				BC_2S_5	2.13	1.98	2.05
IBC-25	Standard		*MLB2*			*MLB5*		BC_2S_5	0.93	1.25	1.09
IBC-235	Standard		*MLB2*	*MLB3*		*MLB5*		BC_3S_5	1.30	0.59	0.94
IBC-0	Standard							BC_2S_5	1.38	1.89	1.64

[a] IBC line names reflect their QTL composition (i.e., IBC-1246 contains *MLB1*, *MLB2*, *MLB4*, and *MLB6*)

[b] Leaf type classified as standard- (>40 cm^2) or little-leaf (30–40 cm^2; Staub et al. 1992)

[c] QTL names are from Table 1

[d] Lsmeans for Hancock, Wisconsin, Arlington, Wisconsin, and combined locations, where LSD's ($\alpha = 0.05$) are 0.80, 0.82, and 0.57, and Coefficients of variation (CV) are 23.8%, 15.3%, and 20.3%, respectively

Table 4 Means comparisons to determine the effects of specific quantitative trait loci (QTL) in inbred backcross (IBC) lines of cucumber

Means comparison[a]	Comparison	Test hypothesis[b]			
		QTL effect	Background	*P*-value[c]	QTL × loc *P*-value[d]
IBC-1246 vs. IBC-146	A	*MLB2*	*MLB4*	0.0807	0.0934
IBC-12456 vs. IBC-1246	B	*MLB5*	*MLB2* & *MLB4*	0.7707	0.0854
IBC-12356 vs. IBC-1236	C	*MLB5*	*MLB2* & *MLB3*	0.4638	0.2363
IBC-12356 vs. IBC-1356	D	*MLB2*	*MLB3* & *MLB5*	0.1308	0.1740
IBC-23 vs. IBC-0	E	*MLB2* & *MLB3*		0.1116	0.2026
IBC-235 vs. IBC-23	F	*MLB5*	*MLB2* & *MLB3*	<0.0001	0.2926
IBC-25 vs. IBC-0	G	*MLB2* & *MLB5*		0.0368	0.7105
IBC-235 vs. IBC-25	H	*MLB3*	*MLB2* & *MLB5*	0.5767	0.0513

[a] The means (over both locations) of the two lines in the means comparison column were tested by single degree of freedom contrasts with the null hypothesis as equal means. IBC line names reflect their QTL composition (see Table 3)

[b] Null hypothesis, where adding the QTL in QTL effect column to the QTL already present in the background column has no effect on the number of lateral branches

[c] The *P*-value of the means comparison

[d] The *P*-value of the QTL by location (loc; Hancock and Arlington, Wisc.) interaction

Twenty-four seedlings from each of the 50 BC_2S_1 and BC_3S_1 families were genotyped using markers linked to QTL for MLB (Table 2). Since none of the individuals contained all six QTL, the marker information was used to identify individuals within a leaf type with complementing QTL compositions that could be crossed to provide progeny segregating for several QTL. A total of 11 little-leaf and nine standard-leaf crosses were made, and 10–20 seeds of each cross were evaluated at Hancock for MLB to

identify 10 (five little-leaf and five standard-leaf) crosses where the marker genotype (parental types) was confirmed by progeny branch number. Progeny (2–96 individuals) from each of these 10 crosses were genotyped to select plants that possessed varying QTL arrays, and then these selections were genotyped and selfed for four to five generations to produce $BC_2S_{5/6}$ or $BC_3S_{5/6}$ lines differing in the number of QTL associated with MLB in both standard- and little-leaf backgrounds (Table 3). The lines are denoted as IBC lines even though they differ slightly from the original description of IBC lines (Wehrhahn and Allard 1965) in that crosses were made between BC_2S_1 and BC_3S_1 families rather than strictly selfing BC_2S_1 and BC_3S_1 families to the $BC_2S_{5/6}$ or $BC_3S_{5/6}$ generation.

Molecular marker analysis

Nine molecular markers and the *ll* gene were employed to track the introgression of QTL into IBC lines (Table 2). All markers were from the Fazio et al. (2003b) genetic linkage map, except AJ6SCAR and M8SCAR, which were SCAR markers (Robbins 2006) converted from RAPD markers mapped by Fazio et al. (2003b). Markers flanking each QTL were utilized, where available, to reduce the chance of selecting individuals with marker-QTL recombination events. Leaf tissue collection, DNA extraction, and subsequent polymerase chain reaction (PCR) amplification and agarose gel electrophoresis were performed according to Fazio et al. (2003b).

Open-field evaluation of IBC lines for MLB

To examine individual QTL effects, QTL number, and growing environment on lateral branch production, IBC lines were evaluated in an open-field trial in the summer of 2005 at three within-row spacings in two locations. Seeds were sown in a greenhouse in Madison, Wisc. on June 11 and June 14, 2005, and then transplanted on June 27 and June 29 to the University of Wisconsin Agricultural Research Stations at Hancock and Arlington [Plano silt loam (Typic Argiudoll)], Wisc, respectively. Each location was arranged in a split-plot design with four replicates of spacing (whole plot factor) in randomized complete blocks, with the IBC lines completely randomized as subplots with 10 plants per subplot. Plots were arranged in single rows with 1.5 m between rows and 10, 15, or 20 cm between plants, corresponding to approximately 66,700, 44,400, or 33,300 plants ha^{-1}, respectively. Lateral branch number (at least three internodes in length) of each plant was recorded at or after anthesis at the first 10 nodes of the mainstem.

Statistical analysis

Branching data were checked for normality using PROC UNIVARIATE of SAS (2003). Although the data were slightly skewed at the tails of the distribution, the deviation was not enough to warrant data transformation. The data were then analyzed by analysis of variance using PROC GLM of SAS (2003) to test for the main effects and interactions of location, spacing, leaf type, and entry (IBC lines) nested within leaf type. All effects, except blocks, were considered fixed effects. Location was considered a fixed effect because the two locations chosen did not represent a sampling of all cucumber growing environments, and inferences were desired for Hancock, specifically, because it was the location originally used for the detection of MLB QTL (Serquen et al. 1997b; Fazio et al. 2003b). The two degrees of freedom for spacing were partitioned into single degree of freedom contrasts within analyses of variance to test for the linear (regression) and residual effects of spacing. The regression coefficients of MLB on spacing were then obtained using PROC REG of SAS (2003). Least squares means (lsmeans) are presented because of missing plots [22 of 288 (7.6%)]. To test for effects of specific QTL, comparisons were made between specific IBC lines that differed in a single QTL (i.e., 12 IBC lines allowed for 11, single degree of freedom contrasts; Table 4). For example, all little-leaf lines in Table 3 have *MLB1* and *MLB6*. In addition, IBC-146 has *MLB4*, IBC-1246 has *MLB2* and *MLB4*, and IBC-12456 has *MLB2*, *MLB4*, and *MLB5*. Thus, IBC-146 and IBC-1246 have all MLB QTL in common, except for *MLB2*. The addition effect of *MLB2*, therefore, is tested by comparing the means of IBC-146 and IBC-1246 (Comparison A; Table 4). Likewise, the effect of adding *MLB5* to *MLB2* and *MLB4* is tested by Comparison B (IBC-12456 vs. IBC-1246).

Results

The main effect of location on MLB was not significant ($P = 0.328$) while all other main effects (spacing, leaf type, and entry nested within leaf type) were highly significant ($P < 0.001$). The interactions of location with spacing and leaf type were not significant, but the location by entry interaction was at the $\alpha = 0.05$ significance level ($P = 0.055$). Therefore, lsmeans of MLB for each IBC line are presented separately and combined over both locations (Table 3). Although the spacing by entry interaction was not significant, the spacing by leaf type interaction was only marginally non-significant ($P = 0.069$). Therefore, the effect of spacing on MLB is presented separately for each leaf type (Fig. 1). Both the linear and residual effects of spacing were significant in IBC lines of both leaf types (Fig. 1). The lsmeans for MLB were 6.34, 7.79, and 7.92 (little-leaf lines) and 0.91, 1.58, and 1.79 (standard-leaf lines) branches per plant at within-row spacings of 10, 15, and 20 cm, respectively ($LSD_{0.05} = 0.30$).

The results of the means comparisons designed to test the effect of adding a specific QTL (see Materials and methods) are presented in Table 4. None of the six means comparisons nor the QTL by location interactions for little-leaf lines (Comparisons A–D) were significant at the $\alpha = 0.05$ level (Table 4). In the standard-leaf lines (Comparisons E–H), the effect of *MLB3* when evaluated in combination with *MLB2* (Comparison E) or alone (Comparison H), was not significant. In contrast, the effect of *MLB5* was significant when alone (Comparison F) or with *MLB2* (Comparison G). In both cases, the MLB mean decreased with the addition of *MLB5* (Fig. 2). Only one QTL by location interaction tested in the standard-leaf lines was near the $\alpha = 0.05$ significance level (Comparison H, $P = 0.051$).

The number of lateral branches in the little-leaf IBC lines did not generally change when the number of QTL was increased, while the number of branches of the standard-leaf lines tended to decrease with increasing numbers of QTL (Fig. 2). In all cases, increasing the number of QTL in the little-leaf background from three to four or from four to five did not significantly increase the number of lateral branches. In contrast, in standard-leaf plants, the

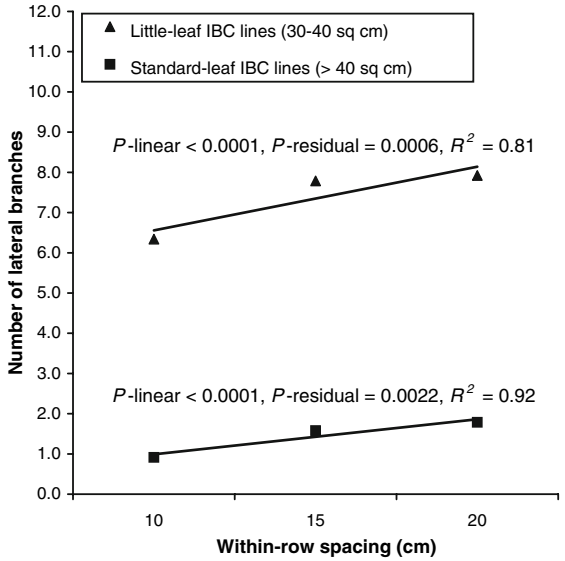

Fig. 1 The effect of plant density on the number of lateral branches in cucumber inbred backcross (IBC) lines of two leaf types (little- and standard-leaf). The linear and residual *P*-values and R^2 of each leaf type are presented above the line. Within-row spacings of 10, 15 and 20 cm correspond to plant densities of 66,700, 44,400, and 33,300 plants ha^{-1}, respectively

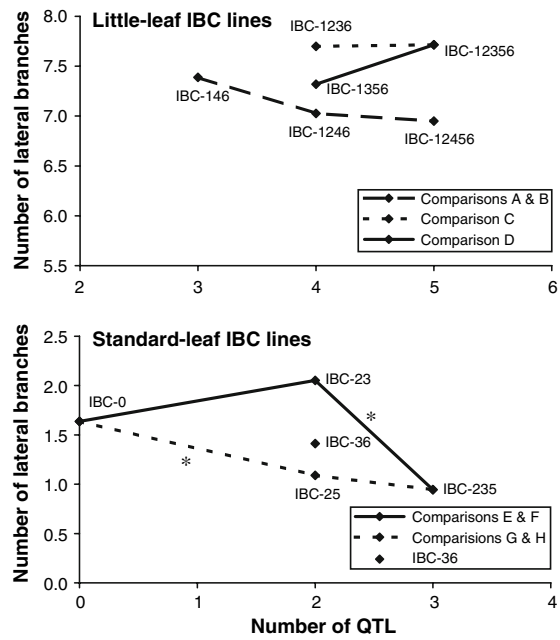

Fig. 2 The effect of increasing the number of quantitative trait loci (QTL) on the number of lateral branches in two leaf types (little- and standard-leaf) of cucumber as determined by comparisons of inbred backcross (IBC) lines (Table 3). Lines indicate the incremental addition of QTL, and the comparisons in the legends refer to means comparisons performed (Table 4). Asterisks (*) indicate significant means comparisons ($P < 0.05$)

increase from zero to two QTL either did not change (IBC-0 to IBC-23) or decreased (IBC-0 to IBC-25) the number of branches. Similarly, the number of lateral branches decreased (IBC-23 to IBC-235) or did not change (IBC-25 to IBC-235) when a third QTL was added.

Discussion

To characterize the effects of combining individual QTL for MLB in cucumber, marker-QTL relationships for MLB, previously identified (Serquen et al. 1997b; Fazio et al. 2003b), and subsequently verified by MAS (Fazio et al. 2003a; Fan et al. 2006; Robbins 2006), were utilized to create IBC lines with varying QTL compositions. Based on the effects estimated from the QTL analyses of Serquen et al. (1997b) and Fazio et al. (2003b), the alleles of the highly-branched H-19 parent at all but one QTL (*MLB3*) should contribute to higher lateral branch number (Table 1). Although the estimated effects of each QTL are dissimilar, incrementally combining the Gy-7 allele of *MLB3* with the H-19 allele at all other QTL should predictably increase the number of lateral branches in either little- or standard-leaf lines under an additive inheritance model with no epistasis.

The number of lateral branches in H-19 or *C. s.* var. *hardwickii* derived germplasm was not affected by environment (Georgia and Wisconsin; Serquen et al. 1997a) or planting date (early and late; Fredrick and Staub 1989) in previous studies. Additionally, in Gy-7 × H-19 derived populations the same four QTL were identified in three different environment/year combinations (Wisconsin in 1999 and 2000 and Utah in 1999; Fazio et al. 2003b). The expression of MLB was not significantly different in the two locations tested in this study (Table 3), as evidenced by the non-significant location main effect and QTL by location interactions (Table 4). These results indicate that the MLB habit can be relatively stable across environments. However, Fazio et al. (2003b) identified a QTL specific to Hancock, Wisc. (LOD 2.7–3.0 in both years), and seven other QTL (LOD 2.8–6.1) unique to a single environment and the effects of several QTL widely varied across environments. These results, coupled with an estimate of narrow-sense heritability of 0.48 for MLB (Serquen et al. 1997a), indicate that MLB can be influenced by the environment to some degree. Indeed, the number of lateral branches in Gy-7 × H-19 derived populations varied across years (López-Sesé and Staub 2002) and planting dates (Robbins 2006) when grown at Hancock, Wisc. This apparent disparity suggests that selection for MLB in one environment under a given plant density will increase the number of lateral branches, but selection should be practiced in several growing environments to optimize gain from selection for wide adaptation.

Our finding that the number of lateral branches decreased with increased plant density (Fig. 1) is consistent with previous results in *C. s.* var. *hardwickii* germplasm (Fredrick and Staub 1989). The effect of spacing was similar for all lines and locations examined herein. The highly significant residual effect of spacing on MLB suggests their relationship is not linear (Fig. 1). The decrease in the number of branches when the spacing between plants was reduced from 20 to 15 cm was smaller than when spacing was reduced from 15 to 10 cm in both leaf types. This difference may be slightly less pronounced for the little-leaf lines than for standard-leaf lines (i.e., spacing by leaf type interaction, $P = 0.069$; Fig. 1). This reduction in number of lateral branches at higher plant densities, coupled with the positive correlation of branching with fruit per plant, suggests that the optimal plant density for yield of highly branched genotypes may be lower than that of unbranched genotypes (100,000–200,000 plants ha^{-1}) in machine harvest operations (Wehner 1989; Staub et al. 1992). Thus, the relationship of branching and yield must be evaluated in highly branched genotypes at plant densities above those of this study (i.e., 44,400–100,000 plants ha^{-1}) to determine the optimal density.

The pyramiding of QTL using IBC lines has allowed for further characterization of individual QTL involved in MLB. In general, increasing the number of QTL for MLB did not increase the number of lateral branches (Fig. 2). In fact, increasing the number of QTL in the standard-leaf lines generally decreased the number of branches. Although a similar decrease was not observed in the little-leaf lines, combining the same QTL did not increase the number of lateral branches. Fazio et al. (2003b) estimated that the effects of individual QTL are not equal (Table 1), and that a single QTL, *MLB1*, mapped near the *ll* gene and had the greatest effect on MLB

($R^2 = 32.4\%$; Table 1). Because *ll* was used as a marker for the introgression of *MLB1* (Table 2), only little-leaf IBC lines contained this QTL. The effect of this genomic region on MLB in the IBC lines is illustrated by the substantial difference in branch number between the lsmeans of the standard-leaf line with the greatest number of branches (i.e., IBC23, 2.1 branches per plant) and the little-leaf line with the fewest branches (i.e., IBC12456, 7.0 branches per plant) as well as the difference in lsmeans of little-leaf lines compared to standard-leaf lines over multiple spacings (Fig. 1).

In little-leaf lines, the addition of *MLB2* (Comparisons A and D) and *MLB5* (Comparisons B and C) in multiple cases did not increase the number of lateral branches. This result demonstrates that the effects of *MLB2* and *MLB5* are negligible when in the presence of *MLB1*. However, adding *MLB5* in standard-leaf lines either in combination with *MLB2* (Comparison G) or alone (Comparison F), resulted in a decrease in the number of lateral branches. Furthermore, the decrease from both of these comparisons is consistent in both the environments tested, which suggests that *MLB5* has a negative effect on MLB in the standard-leaf IBC lines. This finding is different from the positive effect of MLB5 identified by Fazio et al. (2003b; Table 1). Although the reason for this difference cannot be conclusively determined from this study, there are several plausible explanations. One explanation, based on results of this study, is that genetic background has a large effect on MLB. The differential effect of *MLB5* depending on leaf type demonstrates the epistatic effect that the linkage block containing *MLB1* and the *ll* gene has on enhancing MLB. Thus the expression of MLB depends, to a large degree, on the presence of the genomic region containing *ll* and *MLB1*, which is an important consideration when breeding for MLB in cucumber. Another explanation for the inconsistent results of *MLB5* is that the structure of the population from which a positive effect was identified for *MLB5* (RIL; Fazio et al. 2003b) is different from the IBC lines tested in this study. In any case, the difference between the current study and previous results is intriguing enough to warrant further investigation into *MLB5*.

High-yielding, highly-branched plant types are desirable for once-over machine harvest of pickling cucumber. Based on our results, the most highly-branched individuals will posses *MLB1*, while the selection of *MLB2* and *MLB5* is not as critical for increasing branch number. When selecting *MLB1* and *ll* to increase the number of branches, the cucumber breeder needs to consider that, although little-leaf plants tend to have more branches than those with standard-sized leaves, little-leaf types tend to have poor fruit quality, be monoecious, and flower later, resulting in low first harvest yield, which is undesirable for machine harvest operations (Lower and Edwards 1986; Wehner 1989). One approach to avoid these undesirable characteristics of little-leaf types is to select for MLB in standard-leaf backgrounds. Our results indicate that selection for *MLB5* will not increase MLB in standard-leaf lines. The lsmean of IBC-36 suggests that *MLB3* and *MLB6* may also not increase branch number. Further investigation is needed, however, to verify these results, and to characterize the individual effects *MLB3*, *MLB4*, and *MLB6*. Because of the structure of the IBC lines, the individual effects of these QTL could not be tested in little-leaf or standard-leaf lines because not all possible QTL combinations were present, and none of the lines contained all six (little-leaf lines) or five (standard-leaf lines) QTL.

The specific function of the QTL associated with MLB is largely unknown. Many of the QTL affecting MLB have been mapped near QTL for other traits, such as sex expression, fruit length to diameter ratio, and fruits per plant, and MLB is consistently correlated (either negatively or positively) with these traits (Kupper and Staub 1988; Serquen et al. 1997a; Cramer and Wehner 1998, 1999, 2000a; Fazio et al. 2003b). Several QTL for MLB have been mapped to genes of known function such as the *ll*, determinate (*de*) and female (*F*) loci (Serquen et al. 1997b; Fazio et al. 2003a, b). It is likely that not all QTL mapped to these genes play major roles in branch number determination, but represent pleitropic effects on MLB (Fazio et al. 2003b). Although determinate and/or gynoecious individuals with several branches have been identified in little- and standard-leaf backgrounds, they generally have fewer branches than their indeterminate and/or monoecious counterparts. Furthermore, the simultaneous improvement of MLB and negatively correlated traits (e.g., gynoecy and earliness) using both phenotypic selection and MAS has been largely unsuccessful (Robbins 2006). Thus, even if all the QTL that promote MLB are combined, the expression of MLB will depend upon plant

architecture (e.g., *ll*, *de*, and *F*), genetic background, and growing environment (plant spacing). These observations suggest that some of the factors involved in MLB may be genes whose primary function is not involved in branching, but in the regulation of developmental processes and source (i.e., photosynthetic base) to sink (i.e., fruit) relationships. Therefore, other traits (e.g., leaf type, determinate character, gynoecious sex expression, earliness, and fruit length to diameter ratio) must be considered when breeding for highly branched cucumber phenotypes to improve yield in cucumber.

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